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γ-Caseins Isolated from Milk Samples Typed β-Casein A^1 and A^3

Abstract

γ-Caseins A^1 and A^3 were isolated from cow milk typed A^1 and A^3 with respect to β-casein. Disc gel electrophoretic patterns of γ-A^1, A^2, A^3, and B were compared with corresponding β-casein patterns. The finding that the same relative spacings occur for the polymorphs of both γ- and β-caseins suggests that the amino acid substitutions which differentiate the β-caseins also differentiate the γ-caseins.

Introduction

γ-Casein occurs in two forms, A and B, as determined by disc gel electrophoresis in 4 M urea, pH 9.6 (3). Furthermore, there appears to be a genetic relationship in the synthesis of γ- and β-caseins, since the A types of both occur together as do the B types. No γ-casein has been found in milks typed β-casein C.

Peterson et al. (4, 7) found that β-casein A is subdivided into A^1, A^2, and A^3 types by gel electrophoresis of caseins at acid pH in the presence of urea, and Groves (1) has shown in a similar manner that γ-casein A can likewise be differentiated into three forms. In samples typed β-caseins A^1, A^2, and A^3, corresponding polymorphs of γ-casein can be demonstrated (1). Groves and Gordon (2) isolated and compared the properties of γ- and β-caseins isolated from milk samples typed β-caseins A^2 and B. By the same methods of fractionation we have isolated γ-caseins A^1 and A^3.

Experimental Procedures

γ-Casein A^1 was isolated from a milk sample typed β-casein A^1 which was obtained locally, and γ-casein A^3 was obtained from the milk of a cow homozygous for β-casein A^3 which was received from Hawaii (in the frozen state [1]).

The samples were fractionated as previously described (2). For the first fractionation 12 g casein were dissolved in 0.005 M phosphate, pH 8.3, and applied to a fibrous DEAE-cellulose column at 3 C. A temperature-sensitive (TS) fraction was eluted with the starting buffer while the γ-casein fraction was eluted at 0.02 M phosphate, and finally the β-casein was eluted at 0.10 M phosphate, pH 8.3. The β-caseins were isolated for comparative purposes. The γ- and β-caseins were refractionated by chromatography on microgranular DEAE-cellulose to obtain purified samples.

Results and Discussion

Disc gel electrophoretic patterns at pH 9.6, 4 M urea, of γ-, β-caseins A^1 and A^3 are shown in Figure 1. Under these conditions γ- and β-caseins can be distinguished, but the A^1 and A^3 types of both γ- and β-caseins show the same mobility. At pH 4.3 and 8 M urea (Fig. 2) disc gel electrophoretic patterns show that all the polymorphs of γ- and β-caseins are resolved. The γ-, β-caseins A^2 and B, together with β-casein C samples, are included for comparison. Kopfler et al. (5) attribute the electrophoretic resolution of β-caseins A^1, A^2, and A^3 at acid pH values to variation in their content of histidine; namely, 6, 5, and 4 residues per molecule, respectively.

Figure 3 is an enlargement of the disc gel patterns for the composite of γ- and β-casein types (Fig. 2) in which the bands representing the corresponding polymorphs A^1, A^2, A^3, and B are aligned. In effect, this manipulation of the photograph cancels out the charge differences between γ- and β-caseins. This charge difference is due primarily to variation in phosphorus content. [γ-Casein has 1 atom of phosphorus per molecule; β-casein has 4 or 5 per molecule depending on the type (2).] The finding that electrophoretic bands can be perfectly matched is consistent with the idea that the same differences in amino acids should be found for corresponding polymorphs of γ- and β-caseins. This is true for the A^2 and B types of both γ- and β-caseins in which serine/arginine and histidine/proline substitutions are inferred (2). Determinations of the composition of the A^1 and A^3 types are now in progress.

Gene duplication is often suggested as a mechanism to explain slight biochemical differences in proteins. In fact, Lush (6) suggests that the α_{s1}- and β-casein loci may have arisen by duplication from a common source. Since the two loci are on the same chromosome and closely linked, Lush postulates that the original

[1] We are grateful to Dr. Geoffrey Ashton for this sample taken from a cow in a Holstein herd in Hawaii.

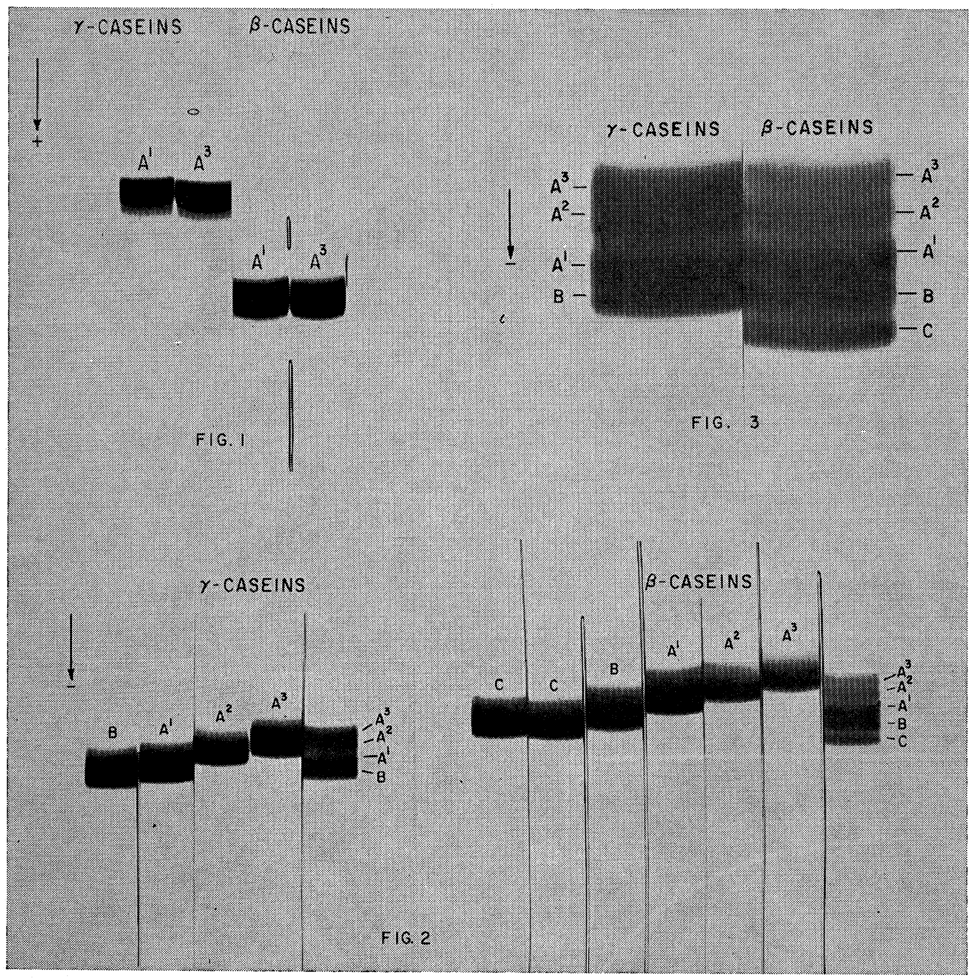

FIG. 1. Disc gel electrophoretic patterns, pH 9.6, 4 M urea, of γ-, β-caseins A^1 and A^3.

FIG. 2. Disc gel electrophoretic patterns, pH 4.3, 8 M urea, of γ-, β-casein polymorphs B, A^1, A^2, A^3, and β-casein C, together with a mixture of γ-casein B, A^1, A^2, A^3 and β-casein C, C, A^1, A^2, A^3 on a single gel. The two β-casein C samples were isolated from a homozygous CC and heterozygous A^2C casein sample.

FIG. 3. An enlargement of the composite disc gel electrophoretic patterns for γ- and β-caseins shown in Figure 2.

divergence was relatively recent in evolutionary history. The finding of similar variants (A^1, A^2, A^3, and B) in both γ- and β-caseins which always appear together (3) suggests that duplication may have been involved in the differentiation of these two systems. It also suggests that the γ- and β-casein divergence may have been more recent than that of the α_{s1}- and β-caseins.

The occurrence of β-casein C is not accompanied by the presence of a similar γ-casein (3). However, as Lush points out, mutants unable to make a recognizable protein may arise from changes that occur following duplication.

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